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Research Article

Assessment of some Immunological Parameters in Sera of Hepatitis C Virus Infected Patients in Babylon – Iraq

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ABSTRACT

In developing nations, the major cause for liver infection that lead to liver fibrosis and cancer is the virus type C. The research has been conducted on 800 individuals assumed to be infected with hepatitis C virus, those individuals have been admitted to AL-Hilla Teaching Hospital ,Central Public Health laboratory, to the possible relation of serum interferon -α, CD56, IL-10 and IL-12 in case infection of virus type C comparison to normal healthy control groups. ELISA assay was used for detection antibodies to hepatitis C virus antigen as well as its immunological response in the subjects which were infected. The result indicated that just 40 from 800 individuals (12-62) years old had HCV positive which represent (5.2%) with high frequency in male(67.3%/), than female(32.6%) groups . The study also show elevated of IL-10 level in all groups of patients compared to the groups of normal healthy controls **(**P<0.05). The highest level was reached to 81.61pg/ml and 90.69pg/ml in male and female respectively. The IL-12 level also revealed a significant increase level in all groups of patients compared to the group of normal healthy controls **(**P<0.05). The highest level for it was reached to 99.25 pg/ml and 125.33pg/ml in male and female respectively. The study also revealed enhancement of IFN- α levels in all groups of patients compared to the groups of normal healthy controls **(**P<0.05) the higher level which was 201.69 pg/ml and 311.71pg/ml male and female respectively. The results also shows increase level of CD56 in all groups of patients compared to the group of normal healthy controls **(**P<0.05). The highest level was reached to 27.984ng/ml and 30.190ng/ml in male and female respectively

Keywords: HCV, IFN-α, CD56, IL-12, IL-10.

INTRODUCTION

The infections of hepatitis C virus (HCV) result in approximately three percent of chronic hepatitis in over seventy percent of infected individuals, whereas twenty to thirty percent of patients recover in spontaneous way[1]. The immune response of the host to hepatitis C virus cause pathogenesis as well as the viral infections. The immune system have the ability of clearing a lot of viruses in the infection's acute phase. Innate immune responses can be considered as the first defense line against viral infections, interferons have the responsibility of activating the cells' antiviral state and for activating and regulating the cellular components related to innate immunity, for example the natural killer cells (NK)[2]. Type I IFNs (including some IFN-β and IFN-α) are developed via cells which have been infected with viruses and through key sentinel cells related to the innate immune system: dendritic cells (DCs) and macrophages. Notably, there is no need for DCs and macrophages to be infected via viruses for producing IFNs. However, they always sample material from outside, such as virus containing remnants related to apoptotic cells as well as intact viral particles. Degradation processes in endosomes then expose viral nucleic acids to recognition via toll-like receptors (TLRs). Type II IFN (IFN-ᵞ) will be produced via NK and natural killer T (NKT) cells as part of innate immune response, and through antigen-specific T cells (CD4+ Th1 and CD8+ cytotoxic T lymphocytes). Virus infections are detected through TLR dependent pathway[3] and the cytosolic pathway, activate via binding the RNA of virus to RNA helicases retinoic acid inducible gene-1 (RIG-1) and melanoma differentiation antigen 5 (Mda5)[4]. Both pathways converge on activation of key transcription factors NF-jB and the interferon regulatory factor (IRF) 3 and 7. Activated NF-jB and IRF3 bind to response elements in promoters of type I and III IFN genes. All IFN types work on inducing an antiviral state through the transcriptional activation of hundreds of genes. The specific genes' set varies between IFNs and target cell type. Generally, IFN-ks and IFN-as work on inducing mainly overlapping genes' set in cells which prompt receptors for IFN-k and IFN-a[5], while IFN-c-induced gene set is more distinct[6]. The number of the genes which have been regulated via IFNs varies between cells. For example, pegylated IFN-a induced (200-300) genes in liver and approximately 2000 genes in peripheral blood mononuclear cells (PBMCs)[7]. NK cells can be considered as large granular lymphocytes account for almost all innate immune cells in the liver of human[8]. Certainly, they are considerably increased in liver in

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Table 1: Assessment the IL-10 concentrations (Pg/ml) in control and patients according to sex.

Age (Year)	Samples	IL-10 (Pg/ml) (Mean ±S.D)	
		Male	Female
12-22	Control	15.23±4.71	16±2.80
	samples	*81.61±12.59	*74.52±9.26
23-33	Control	10.35±2.71	13.5±2.86
	samples	*77.42±25.73	*90.69±7.88
34-44	Control	12±3.68	19.67±8.38
	samples	*69.57±34.33	*77.67±28.32
45-55	Control	20±7.60	10.22±3.53
	samples	*50±19.10	*80.79±3.24
52-62	Control	11.2±8.11	13.0±1.62
	samples	*53.39±37.85	*55.22±17.33
$LSD_{(0.05)}$	7.76		

* ($P \leq 0.05$)

Table 2: Assessment the levels of IL-12 (Pg/ml) control and patients according to sex.

Age (Year)	Samples	IL-12(Pg/ml)	
		Male	Female
12-22	Control	30.26±0.52	22.11±7.5
	Samples	*81.14±19.13	*99.31±27.1
23-33	Control	18.63±2.11	17.01±5.44
	Samples	*90.14±24.71	*125.33±17.15
34-44	Control	29.55±2.11	30.21±5.11
	Samples	*100.25±30.55	*101.10±60.0
44-55	Control	14.9±3.1	15±2.81
	Samples	*78.0±41.80	*88±11.67
52-62	Control	18.3±5.33	30.5±4.90
	Samples	*57.11±35.06	*68.9±25.42
$LSD_{(0.05)}$	30.40		

* ($P \leq 0.05$)

Table 3: Levels of IFN- α in of control and patients according to * ($P \leq 0.05$).

Age (Year)	Samples	IFN- α (Pg/ml)	
		Male	Female
12-22	Control	20.5±4.51	14.67±3.09
	Samples	*103.69±19.38	*117.02±21.96
23-33	Control	18.75±3.30	18.5±2.04
	Samples	*201.69±20.19	*311.71±8.16
34-44	Control	22±6.68	18.67±8.38
	Samples	*100.37±16.30	*188.71±23.63
45-55	Control	21.33±3.68	26.33±3.68
	Samples	*89.35±44.52	*169.04±38.59
52-62	Control	23.5±2.04	21.5±2.86
	Samples	*79.32±27.02	*85.38±16.64
$LSD_{(0.05)}$	13.54		

* ($P \leq 0.05$)

comparison with peripheral blood even though this turn out to be particularly obvious in chronic infections of HCV.

Natural killer cells are of high importance in controlling viral infections as they have direct antiviral in addition to regulatory effects[9]. The direct antiviral effects are mediated through direct cytolytic (TRAIL or perforinmediated) or non-cytolytic (IFN-c mediated) effector functions[10]. The exact role regarding the immune response in individuals experiencing HCV, especially the relation between the course of HCV and the levels of inflammatory/regulatory cytokines is still uncertain. That such cytokines could activate distinct patterns of immunopathological or protective responses and they are involved in the establishment or clearance of chronic HCV infection[12]. Yet, the balance related to regulatory and proinflammatory cytokines seems to be of high importance in defining the course of HCV infection[5]. Cytokines are of high importance in the differentiation, maturation, and functional activation of immune cells[13]. Cytokines are created via multiple types of cells like natural killer cellsl macrophages CD4+T cells and CD8+T cells. Responses will be denoted as Th2-like and Th1-like after the original description of cytokine profiles created via subsets of CD4+T cells[14]. Th1-like responses consist of IL-2, TNF-α, and IFN-γ secretion, also they are needed to generate cytotoxic T lymphocytes and natural killer cell activation throughout the host antiviral immune response. Th2-like responses create IL-10 and IL-4, that support the production of augment antibody and inhibit development of Th1 response, IL-10 is a pleiotropic cytokine created via macrophages T-helper 2 (Th2) cells and B-lymphocytes, and both could be simulating and suppressing the immune response. IL-10 inhibit many immune reactions[15]. Interleukin- 12 (IL-12) can be defined as a major proinflammatory cytokine created primarily via antigen presenting cells because of IFN-γ stimulation and presented with initiation of immune response, this the IL-12 could be regarded as one of the most factors defining the differentiation of Th1 and Th2 ,thus, the present research intended to study the serum expression of inflammatory and immunoregulatory cytokines (IL-10/IL-12) with HCV infection in chronic liver disease and for evaluating their potential role as new biomarkers in chronic inflammation progression resulting in HCC[16].

MATERIAL AND METHODS

Eight-hundred samples of blood each of five ml volume have been gathered aseptically through the use of sterile syringes from individuals have HCV infection . A sterile plan tube has been used for collecting the blood samples, then they have been labelled and incubated at room temperature until clotting, sample have been collected following an centrifugation in Kokusan (Japan), centrifuge at 3000 r.p.m for ten minutes, all samples has been tested for Hepatitis – C antigen (USA) .In addition the samples have been classified in 0.5 ml aliqutes in sterile eppendorf tubes and kept at –24Celsius for further testing, tests included anti-HCV antibody which has been identified via ELISA detection (USA) no more than forty individuals ageing (11- 60) years old provided positive HbsAg results. Yet, the needed information have been fixed depending on certain formula, involving the name, age, gender and residence of the subject. The research consist of 40 samples from healthy subject a control

Table 4: Assessment of CD56 (ng/ml) in control and infection groups according to sex.

Age (Year)	Samples	CD56 (ng/ml)	
		Male	Female
12-22	Control	3.616±0.347	1.649±0.460
	Samples	*27.984±0.692	*29.033±1.709
23-33	Control	1.283±0.246	2.071±0.590
	Samples	*16.528±0.653	*30.190±1.845
34-44	Control	2.522±0.705	3.229±0.861
	Samples	*8.594±2.264	*9.569±1.156
45-55	Control	3.276±0.905	2.062±0.129
	Samples	*15.528±4.091	*11.033±0.374
52-62	Control	32.867±0.236	1.939±0.448
	Samples	*21.340±1.681	*14.835±0.229
$LSD_{(0.05)}$	2.064		

* ($P \leq 0.05$)

group, which categorized to five type :(12 – 22), (23 – 33), (34 – 44), (45 – 55), and (52-62) years old. Statistical analysis: Data have been analyzed statistically through the use of complete randomized design(CRD), LSD and X2 test (Naizi , 2004).

RESULT AND DISCUSSION

The study shows high levels of Interleukin 10 in serum of patients have hepatitis C infection in contrast to the group of controls. The maximum levels of Interleukin 10 in women occur in the age group (23-33 year) was (90.69 Pg/ml) whereas the lowermost levels (55.87Pg/ml) occurred in the age group of (52-62year). Furthermore, the results indicated elevated levels of Interleukin 10 in male at (12-22 year) age group that reached (81.09 Pg/ml), whereas the low levels occurred in the age group of (45-55 year) and it has been (50.19)Pg/ml. differences of Significant in contrast to the controls ($P \leq 0.05$) as seen in table (1). Cytokines are considered to have high importance in regeneration, infection control, viral clearance, inflammation, and fibrosis, also they are implicated in the pathological processes taking place in liver throughout viral infection[12]. So, the presented research has been developed for investigating serum level regarding IL-10 and IL-12 in chronic liver disease and their relation with the disease and for evaluating their potential role as new biomarkers in chronic inflammation progression resulting in HCC[ ]. The concentration of IL-10 was stated to be considerably high in individuals experiencing chronic HCV, also IL-10 could be associated to hepatocarcinogenesis with suppression of immune surveillance[17]. Contrasting results has been noticed in measuring the concentrations of IL-10 in serum samples of individuals experiencing chronic infection of HCV via enzyme linked immunosorbent assay (ELISA). Kakumu et al.[14] showed more spontaneous IL-10 production through peripheral blood mononuclear cells (PBMc) in individuals experiencing CHC and liver cirrhosis than in healthy controls. The concentrations of IL-10 has been higher in individuals suffering HCV, cirrhosis, and HCC, the concentrations are related with the progression of the disease signifying that IL-10 indicates the degree of inflammation in liver and could be associated to the development of HCC. Yet, increased circulating IL-10 was indicated in patients with various tumor types such as resectable HCC[21]. These results may be explained on the basis that the elevated levels of IL-10 in patients with HCC caused by the secretion of IL-10 via tumor cells, in addition to the production at the site of inflammatory changes with activated infiltrating mononuclear cells in the liver[22].

In the presented study, it has been indicated that there are elevated concentrations of IL-12 in sera of patients experiencing hepatitis C in comparison with the group of healthy controls. The maximum level of IL-12 in female were in the age group of (23-33 year) and it has been (125.33 Pg/ml) whereas the lowermost level was in the age group of (52-62 year) that reached (68.9Pg/ml).

The results of the study indicated elevated levels of IL-12 in male. The maximum IL-12 levels in male were in the age group of (34-44 year) and it has been (100.25 Pg/ml), while the lowest level were in the age group of (52-62 year) and it was (57.11Pg/ml). The results indicated considerable differences when compared to the group of controls ($P \leq 0.05$), as shown in Table (2). Interleukin twelve is the first inflammatory marker which is secretion via type of cells in the case of intracellular infection[17]. It is of high importance in the coordination of innate and adaptive immunity. The secretion of IL-12 throughout the infection organize innate responses and determine the duration and type of adaptive immune response. Furthermore, such cytokine induces the production of IFN-γ via macrophages, dendritic cells, T cells and natural killer cells, and promote the differentiation of naive CD4+ T cells into Th1 cells which produce IFN-γ and have a role in cellular immune responses[18].

The level of interferon alpha was elevated show increased in infection groups when compared to controls. Maximum concentration in women occur in of (23-33)years and it has been (311.71Pg/ml), while the lowermost level was (85.38Pg/ml) in age group (52-62 year). Furthermore, the maximum level of IFN- α in male was in the age group of (23-33 year) and it was (201.69 Pg/ml), while the lowermost level was in the age group of (52-62 year) and it has been (79.32Pg/ml). The result showed a considerable differences when compared to controls ($P \leq 0.05$) as shown in table (3). =-he first defense line in the case of viral infection is the immune response that called the innate , interferon are considered as central marker have the task of inducing antiviral state in cells and for activating and regulating cellular components of innate immunity, like the NK cells[6]. IFN-α is produced via cells which have been infected with viruses and through key sentinel cells of innate immune system: DCs and macrophages. The virus infections are sensed via TLR dependent pathway[7,8] and cytosolic pathway, triggered through binding viral RNA to RNA helicases retinoic acid inducible gene-1 (RIG-1) and melanoma differentiation antigen 5 (Mda5)[9,10]. The two pathways converge on the activation related to key transcription factors NF-jB and the interferon regulatory factor (IRF) 3 and 7. Activated IRF3 and NF-jB bind to

response elements in the promoters of type I and III IFN genes.

In addition, the NK cell has been measured in sample of infections group and shows increased concentration of CD56 in women, maximum level were in (23-33 year) and it was (30.190ng/ml), whereas the lowest level has been (11.033 ng/ml) in the age group of (45-55 year). The elevation of CD56 level in male in the age group of (12-22 year), the maximum level has been (27.984ng/ml), whereas the lowest level was in the age group of (45-55 year) and it was (15.528ng/ml) Result indicate considerable differences compared to the group of controls ($P \leq 0.05$) as displayed in table (4).

The major function of natural killer cells is the identifying and killing host cells which were infected with virus, in addition to tumor cells. Their responses, although less specific, are considered to be more rapid than the T cell responses. Different to the T cells, no immunological memory is related with the natural killer cells[20-21]. NK cells are considered as large granular lymphocytes which account for the most of innate immune cells in human liver[22]. Certainly, they are considerably increased in the liver in comparison to peripheral blood even though this becomes particularly obvious in chronic infection of HCV. NK cells are of high importance in controlling viral infections. They have direct antiviral and regulatory effects, and the direct antiviral effects are mediated via direct cytolytic[23].

CONCLUSION

The increased concentration of IL-10 and IL-12 with disease progression and transaminase values could be involved in chronic inflammation progression causing HCC and their evaluation might be utilized as new biomarkers for HCC development.

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